

Amendments to the Claims

Claim 1 (Currently amended): A method for screening animals to determine those more likely to produce larger litters comprising:

obtaining a sample of genetic material from said animal; and

assaying for the presence of a genotype ~~in said animal which is associated with increased litter size, said genotype characterized by the following:~~

a) ~~a polymorphism~~ in the prolactin receptor gene sequence as set forth in SEQ ID NO: 3 or a region thereof in said sample, said polymorphism ~~which is associated with increased litter size;~~
and

selecting an animal possessing a nucleic acid sequence having at least 95% sequence identity to a region of the gene set forth in SEQ ID NO: 3 or a fragment thereof.

Claim 2 (Original): The method of claim 1 wherein said step of assaying is selected from the group consisting of: restriction fragment length polymorphism (RFLP) analysis, heteroduplex analysis, single strand conformational polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE).

Claim 3 (Currently amended): The method of claim 1 wherein said step of assaying for the presence of said polymorphism comprises the steps of:

digesting said genetic material with a- said restriction enzyme that cleaves the prolactin receptor gene in at least one place;

separating the fragments obtained from said digestion;

detecting a restriction pattern generated by said fragments; and

comparing said pattern with a second restriction pattern for the pig prolactin receptor gene obtained by using said restriction enzyme, wherein said second restriction pattern is associated with increased litter size.

Claim 4 (Original): The method of claim 3 wherein said restriction enzyme is AluI.

Claim 5 (Original): The method of claim 3 wherein said restriction enzyme is HinFI.

Claim 6 (original): The method of claim 3 wherein said restriction enzyme is HypCH4IV.

Claim 7 (original): The method of claim 3 wherein said restriction enzyme is MseI.

Claim 8 (original): The method of claim 1 wherein said animal is a pig.

Claim 9 (original): The method of claim 3 wherein said separation is by gel electrophoresis.

Claim 10 (original): The method of claim 3 wherein said step of comparing said restriction patterns comprises identifying specific fragments by size and comparing the sizes of said fragments.

Claim 11 (currently amended): The method of claim 3 further comprising, prior to said digestion the step of amplifying the amount of prolactin receptor said gene or a portion thereof which contains said polymorphism, prior to said digestion step with a forward primer and a reverse primer.

Claim 12 (original): The method of claim 3 wherein said polymorphism is a polymorphic AluI restriction site.

Claim 13 (original): The method of claim 3 wherein said polymorphism is a polymorphic HinFI restriction site.

Claim 14 (original): The method of claim 3 wherein said polymorphism is a polymorphic MseI restriction site.

Claim 15 (original): The method of claim 3 wherein said polymorphism is a polymorphic HypCH4IV restriction site.

Claims 16 (original): The method of claim 12 wherein said restriction site is located in the 3' coding region of the pig prolactin receptor gene.

Claim 17 (original): The method of claim 13 wherein said restriction site is located in the region between exons 8 and 9 of the prolactin receptor gene.

Claim 18 (original): The method of claim 14 wherein said restriction site is located in the region between exons 8 and 9 of the prolactin receptor gene.

Claims 19 (original): The method of claim 15 wherein said restriction site is located in the region between exons 8 and 9 of the prolactin receptor gene.

Claim 20 (currently amended): The method of claim 11 wherein said ~~amplification~~ includes the steps of:
~~selecting a~~ forward and a reverse sequence primer is capable of amplifying a region of said pig prolactin receptor gene which contains a polymorphic AluI, HinFI, HypCH4IV, or MseI site.

Claim 21 (original): The method of claim 20 wherein said forward and reverse primers are selected from and based upon SEQ ID NO: 3.

Claim 22 (original): The method of claim 20 wherein said primers are SEQ ID NO: 4 and SEQ ID NO: 5.

Claim 23 (original): The method of claim 20 wherein said primers are SEQ ID NO: 6 and SEQ ID NO: 7.

Claim 24 (currently amended): The method of claim 22 wherein said forward primer is selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:6 and said reverse primer is selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:6 7.

Claim 25 (original): The method of claim 22 wherein said primer set comprise SEQ ID NO:1 and SEQ ID NO:2.

Claim 26 (currently amended): The method of claim 20 wherein said primers are selected from the group consisting of SEQ ID NO:8 and 9; SEQ ID NO:10 and 11₃, and SEQ ID NO:12 and 13.

Claim 27 (original): A method for identifying a genetic marker for litter size in animals comprising the steps of:

breeding male and female animals of the same breed or breed cross or derived from similar genetic lineages;

determining the number of offspring produced by each female animal;

determining the polymorphism in the prolactin receptor gene of each female animal; and

associating the number of offspring produced by each female animal with said polymorphism thereby identifying a polymorphism for pig litter size.

Claim 28 (original): The method of claim 27 further comprising the step of selecting animals for breeding which are predicted to have increased litter size by said marker.

Claim 29 (original): The method of claim 27 wherein said analysis comprises digestion of PCR amplified DNA with the restriction enzyme selected from the group consisting of AluI, HinFI, HypCH4IV, and MseI.

Claims 30-34 (cancelled)

Claim 35 (original): A DNA sequence from the pig prolactin receptor gene 3' translated and nontranslated region, said sequence consisting of SEQ ID NO:3.

Claim 36 (currently amended): A method for screening pigs to determine those more likely to produce larger litters, and/or those less likely to produce larger litters, which method comprises of the steps:

determining the alleles of prolactin receptor present in a pig having SEQ ID NO: 3;

determining the alleles of other markers for genes known to affect litter size; and

selecting for animals with favorable combinations of alleles and against those carrying unfavorable combinations.

Claim 37 (currently amended): The method of claim 36 wherein the determination of prolactin receptor alleles comprises determining the presence of at least one allele associated with at least one DNA marker linked either directly or indirectly to a region of the gene set forth in SEQ ID NO: 3 or a fragment thereof ~~prolactin receptor~~.

Claim 38 (original): The method as claimed in claim 36 wherein the DNA marker is a microsatellite.

Claim 39 (original): The method as claimed in claim 36 wherein the DNA marker is SW1305, S0077, S0006, SW2411, SW1035 and S0111.

Claim 40 (currently amended): A method of screening animals to determine those more likely to produce larger litters comprising:
obtaining a biological sample from said animal; and
assaying for the presence of a genotype in said animal which is associated with increased litter size, said genotype characterized by a restriction fragment pattern, said pattern when compared to a second restriction pattern is known to have or not have a desired marker, wherein the presence of said marker by a polymorphism in the prolactin receptor gene wherein said polymorphism identifiable by a PCR protocol selected from the following: is indicative of an animal more likely to produce larger litters.

~~amplification with four contiguous bases from each of SEQ ID NO:8 and SEQ ID NO:9
and HinFI digestion,~~

~~amplification with four contiguous bases from each of SEQ ID NO:12 and SEQ ID
NO:13 and MseI digestion;~~

~~amplification with four contiguous bases from each of SEQ ID NO:1 and SEQ ID NO:2
and AluI digestion;~~

~~amplification with four contiguous bases from SEQ ID NO:10 and SEQ ID NO:11 and
HpyCH4IV digestion.~~

Claim 41 (new): The method of claim 40 wherein the assaying step comprises amplifying with a forward primer and a reverse primer a region of the prolactin receptor gene containing said marker.

Claim 42 (new): The method of claim 41 wherein said forward primer is SEQ ID NO: 1 and said reverse primer is SEQ ID NO: 2.

Claim 43 (new): The method of claim 41 wherein said forward primer is SEQ ID NO: 8 and said reverse primer is SEQ ID NO: 9.

Claim 44 (new): The method of claim 41 wherein said forward primer is SEQ ID NO: 10 and said reverse primer is SEQ ID NO: 11.

Claim 45 (new): The method of claim 41 wherein said forward primer is SEQ ID NO: 12 and said reverse primer is SEQ ID NO: 13.

Claim 46 (new): The method of claim 40 wherein said marker is AluI.

Claim 47 (new): The method of claim 40 wherein said marker is HinFI.

Claim 48 (new): The method of claim 40 wherein said marker is HypCH4IV.

Claim 49 (new): The method of claim 40 wherein said marker is MseI.

Claim 50 (new): The method of claim 40 wherein said restriction fragment pattern is characterized by a 124 bp fragment, a 110 bp fragment, a 79 bp fragment, a 77 bp fragment, and a 67 bp fragment.

Claim 51 (new): The method of claim 40 wherein said restriction fragment pattern is characterized by a 103 bp fragment, an 86 bp fragment, and a 17 bp fragment.

Claim 52 (new): The method of claim 40 wherein said restriction fragment pattern is characterized by the pattern as shown in Figure 7.

Claim 53 (new): The method of claim 40 wherein said restriction fragment pattern is characterized by a 281 bp fragment and a 140 bp fragment.

Claim 54 (new): A method for identifying a marker correlated with litter size comprising the steps of:

obtaining a sample of genetic material from an animal, said sample comprising a prolactin receptor gene;

assaying said prolactin receptor gene presented in said sample for a polymorphism;

correlating whether a statistically significant association exists between said polymorphism and litter size in an animal of a particular breed, strain, population, or group whereby said animal can be characterized for said marker.

Claim 55 (new): The method of claim 54 wherein said animal is a pig.